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An Alternative Approach to Non-Log-Linear Thermal Microbial Inactivation: Modelling the Number of Log Cycles Reduction with Respect to Temperature**

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Summary

A mathematical approach incorporating the shoulder effect during the quantification of microbial heat inactivation is being developed based on »the number of log cycles of reduction« concept. Hereto, the heat resistance of *Escherichia coli* K12 in BHI broth has been quantitatively determined in a generic and accurate way by defining the time t for x log reductions in the microbial population, *i.e.* t_{xD}, as a function of the treatment temperature T. Survival data of the examined microorganism are collected in a range of temperatures between 52–60.6 °C. Shoulder length S_l and specific inactivation rate k_{max} are derived from a mathematical expression that describes a non-log-linear behaviour. The temperature dependencies of S_l and k_{max} are used for structuring the $t_{xD}(T)$ function. Estimation of the $t_{xD}(T)$ parameters through a global identification procedure permits reliable predictions of the time to achieve a pre-decided microbial reduction. One of the parameters of the $t_{xD}(T)$ function is proposed as »the reference minimum temperature for inactivation«. For the case study considered, a value of 51.80 °C (with a standard error, SE, of 3.47) was identified. Finally, the time to achieve commercial sterilization and pasteurization for the product at hand, *i.e.* BHI broth, was found to be 11.70 s (SE=5.22), and 5.10 min (SE=1.22), respectively. Accounting for the uncertainty (based on the 90 % confidence intervals, CI) a fail-safe treatment of these two processes takes 20.36 s and 7.12 min, respectively.

Key words: predictive microbiology, thermal processing, inactivation kinetics, modelling log cycles of microbial reduction, *Escherichia coli*

Introduction

Quantifying the microbial destruction that takes place during a thermal treatment of a food product or a food model system is one of the issues covered by the discipline *predictive microbiology*. Suitable kinetic parameters and mathematical models are the tools for comparing microbial inactivation data and for designing heat-processing requirements to ensure the microbial safety and quality of a food product. These tools could further contribute to the microbial aspect of Hazard analysis and critical control points system (HACCP) by identifying: (*i*) microorganisms that can be potential hazards (*e.g.* growth/no growth models can show which microorganisms will grow on a product (*1*)), and (*ii*) steps at which critical control can be achieved (*e.g.* models de-

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scribing the impact of different process conditions). Additionally, they can be used for specifying: (*i*) limits of a process in order to avoid emergence of other hazards (*e.g.* performing microbial predictions at different combinations of processing conditions), and (*ii*) corrective actions if a loss of control occurs at a critical control point (CCP) (*e.g.* design corrective steps based on the impact of the process deviation on the change in microbial population) (*2*). Predictive microbiology can also be considered as a key element for the domain of exposure assessment in the context of Quantitative Risk Assessment (as a part of Food Safety management) by estimating the prevalence and levels of microbial contamination of the food product at the time of consumption (*3,4*).

The primary model, describing the response of the microbial load as function of the time, is traditionally chosen as being log-linear. Similarly, the secondary model for microbial inactivation, describing the relation between the rate of inactivation and temperature, is also log-linear. In this model the D_{ref} (decimal reduction time at reference temperature) and the z (thermal resistance constant) values are estimated. The excellent safety record of the last 80 years of the canning industry has proved the model value for high temperature treatments at a sterilisation level. Nevertheless, deviations from log-linearity have also been observed for 80 years (as stressed in (*5*)), and they are a common case in mild heat treatments and emerging non-thermal technologies. These deviations could lead either to over-processing or under-processing, resulting in safety or spoilage problems (*6*). Primary models that can cope with deviations of log-linearity are developed to predict inactivation more precisely (*e.g.* (*7,8*)). The use of these models requires the development of new secondary modelling approaches (describing the response of primary model parameters to changes in one or more environmental conditions) which, when necessary, have to replace classical D_{ref} and z-values.

This observation was also raised in one of the recent research summits of IFT, the Institute of Food Technologists (January 14–16, 2003, Orlando), entitled »Kinetic models for microbial survival during processing«. It was suggested that »the performance of food preservation processes should be communicated in terms of the number of log cycles of reduction that the process is expected to deliver for the microorganism of concern rather than the *D*-value« (*9*).

Therefore, *D*-values should be used with great caution in order to avoid indiscriminate application to the cases they are not appropriate for (*10*). The use of a *D*--value for an acknowledged log-linear section of a survival curve, also noted as *AsymD*, is acceptable and can be found in a lot of studies in the literature (*11–13*). Nonetheless, the previously mentioned studies also illustrate the need for identifying additional parameters for the accurate description of the non-log-linear inactivation kinetics.

The objective of the present study is to quantitatively determine the heat resistance of a model microorganism in a generic and accurate way by defining the parameters of an alternative concept for thermal microbial inactivation. This concept denoted as t_{xD}, and first introduced by Buchanan *et al.* (*14*), describes the time t required for x log reductions in the microbial population (or x D) as a function of the treatment temperature T. Global identification, through incorporation of secondary model structures into the chosen primary model, of the parameters of the t_{xD} function is the main outcome of this research, enhancing the reliable use of non-log-linear inactivation curves that describe microbial heat resistance. Finally, a microbial interpretation of one of the $t_{xD}(T)$ parameters is elaborated upon and the time to achieve commercial sterilization and pasteurization for the case study at hand is calculated.

Materials and Methods

In our work, *E. coli* K12 MG1655 strain was chosen as a surrogate for the food-borne pathogen *E. coli* O157:H7. Survival data of early stationary phase cultures as described in previous research (*13,15*) were used. These experimental data (partially duplicated) were generated at temperatures of 52, 54, 54.6, 55, 56.6, 58.6 and 60.6 °C.

The dynamic sigmoidal-like model of Geeraerd *et al.* (*7*) was used to describe the microbial inactivation of *Escherichia coli* K12 MG1655. As no tailing effect was observed in the experimental data at hand, the model was applied in its reduced version by omitting the N_{res} parameter. This parameter denotes the number of cells in a subpopulation of the total population N (in CFU/mL) that are more resistant to the thermal treatment (the tailing effect was originally incorporated in the model of Geeraerd *et al.* (*7*), based on the arguments proposed by Cerf (*5*)).

The reduced version of the model has one parameter k_{max} (1/min), the maximum specific inactivation rate, and two states, *i.e.* C_c (units/cell), the number of hypothetical protective (or critical) components inside or surrounding the cells that induce a shoulder behaviour related to the physiological state of the cells, and N (CFU/mL), the microbial load:

$$\frac{dN}{dt} = -k_{max} \cdot \left(\frac{1}{1 + C_c} \right) \cdot N \qquad /1/$$

$$\frac{dC_c}{dt} = -k_{max} \cdot C_c \qquad /2/$$

The structure of this model can be described as follows. The first factor at the right-hand side of Eq. 1 models the log-linear part of the inactivation curve and is equivalent to the classical first-order inactivation kinetics. The second factor describes the shoulder effect and is based on the hypothesis of the presence of a pool of protective components around or in each cell (*16*). Gradually, this pool is destroyed. In case of a shoulder (*i.e.* a large number of protective components is present), $1/(1+C_c(0))$ (with $C_c(0)$ the value of C_c at time zero) takes on a small (positive) value. Towards the end of the shoulder region $1/(1+C_c(t))$ becomes (approximately) equal to one, due to the component C_c undergoing heat inactivation that follows a first-order relationship (see Eq. 2). The chosen model structure (Eqs. 1 and 2) has already been tested with data of different microorganisms: *Listeria monocytogenes* and *Lactobacillus sakei* (*7*) during a mild thermal inactivation, *Monilinia fructigena* and

Botrytis cinerea (*17*) during a pulsed white light treatment, *Salmonella enterica* and *L. monocytogenes* for studying the Acid Tolerance Response (ATR) (*18*), *L. monocytogenes* in a pH modified chicken salad during cold storage (*19*), and for the thermal inactivation of different spores (*Bacillus pumilus*, *B. cereus*) and vegetative bacteria (*Clostridium botulinum*, *L. innocua*) (*20*). In all cases the model yielded accurate descriptive characteristics.

For static conditions the model can be written as follows, making use of the relationship between $C_c(0)$ and S_l (shoulder length/min), *i.e.* $C_c(0) = \exp(k_{max} \cdot S_l) - 1$ (*21*):

$$N(t) = N(0) \cdot e^{(-k_{max} \cdot t)} \cdot \frac{e^{(k_{max} \cdot S_l)}}{1 + (e^{(k_{max} \cdot S_l)} - 1) \cdot e^{(-k_{max} \cdot t)}} \qquad /3/$$

$N(0)$ denotes the microbial population at time zero (in CFU/mL). The log format of the model (which is needed further in this research) is as follows:

$$\log(N(t)) = \log(N(0)) - \frac{k_{max} \cdot t}{\ln(10)} + \frac{k_{max} \cdot S_l}{\ln(10)} - \log(1 + e^{(k_{max} \cdot S_l)} - 1) \cdot e^{(-k_{max} \cdot t)} \qquad /4/$$

The number of log cycles of reduction is described by using the concept of t_{xD} in which the time to elapse the shoulder length is incorporated (*14*,*22*). Standard errors of the parameters of Eq. 4 are obtained by calculating the square root of the diagonal elements of the asymptotic variance-covariance matrix (*23*).

$$t_{xD} = S_l + x \cdot AsymD \qquad /5/$$

Herein, t_{xD} is the time required for x log reductions in the microbial population (or x D), $AsymD$ (min) denotes the asymptotic decimal reduction time at temperature T (°C) (*13*). The $AsymD$ describes the negative inverse of the slope of the log-linear part of the sigmoidal-like model in use. Therefore, $AsymD = \ln(10)/k_{max}$. For examples on the use of the $AsymD$-value with respect to different environmental factors see Juneja *et al.* (*11*,*12*) and Valdramidis *et al.* (*13*).

Table 1. Estimated microbial parameters and their standard errors using the primary inactivation model (Eq. 4)

T/°C	S_l/min	k_{max}/(1/min)
52	48.22±12.11	0.03±0.01
54	22.48±3.73	0.13±0.006
54.6	12.17±1.78	0.18±0.007
55	8.43±2.89	0.26±0.02
56.6	8.63±0.54	0.71±0.02
58.6	3.64±0.19	2.10±0.11
60.6	1.19±0.19	4.53±0.29

Results

In order to develop a function of t_{xD} with respect to temperature the two factors of Eq. 5 have to be identified. Firstly, primary fitting (Eq. 4) of the microbial data resulted in estimating the inactivation parameters of the primary model, *i.e.* k_{max} and S_l for all the examined temperatures (Table 1). As depicted in Fig. 1, the evolution of the two parameters can be described from the following expressions:

$$S_l(T) = \frac{a}{T - b} \qquad /6/$$

$$\ln k_{max}(T) = c \cdot T - d \qquad /7/$$

When a hyperbolic (Eq. 6) and a linear function (Eq. 7) are fitted for these two cases, good fitting capacity (Fig. 1) is derived, justifying the choice of the above modelling structures. The temperature T in Eq. 6 is considered a factor influencing the shoulder region of the microbial inactivation. Similar observations are also available in the literature (*24*,*25*). Additionally, when T lowers towards b in Eq. 6 the shoulder length reaches infinity. The fitting capacity was evaluated by estimating the adjusted coefficient of multiple determination,

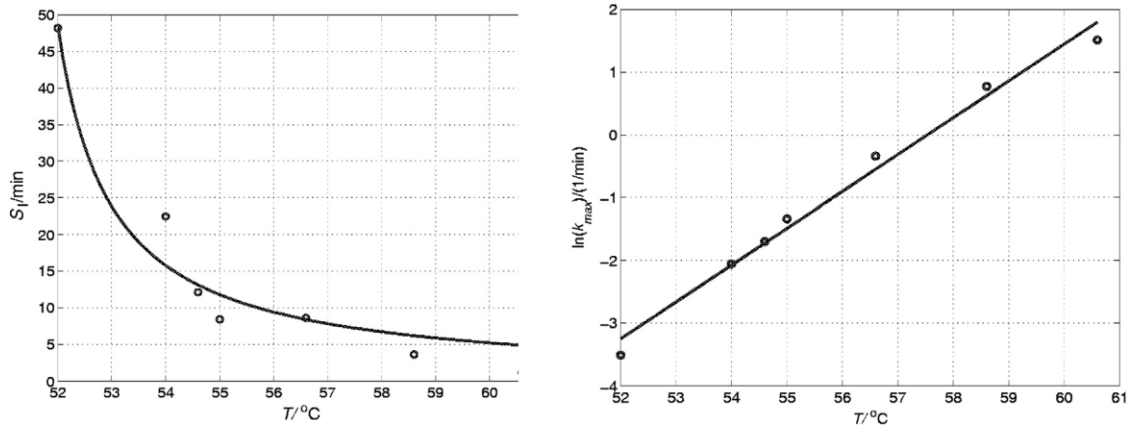

Fig. 1. Evolution of the specific inactivation rate and of the shoulder length estimated from the primary inactivation model (Eq. 4) with respect to the inactivation temperature. The fitting capacity of Eqs. 6 and 7 is R^2_{adj}=0.94 (left plot) and R^2_{adj}=0.98 (right plot), respectively

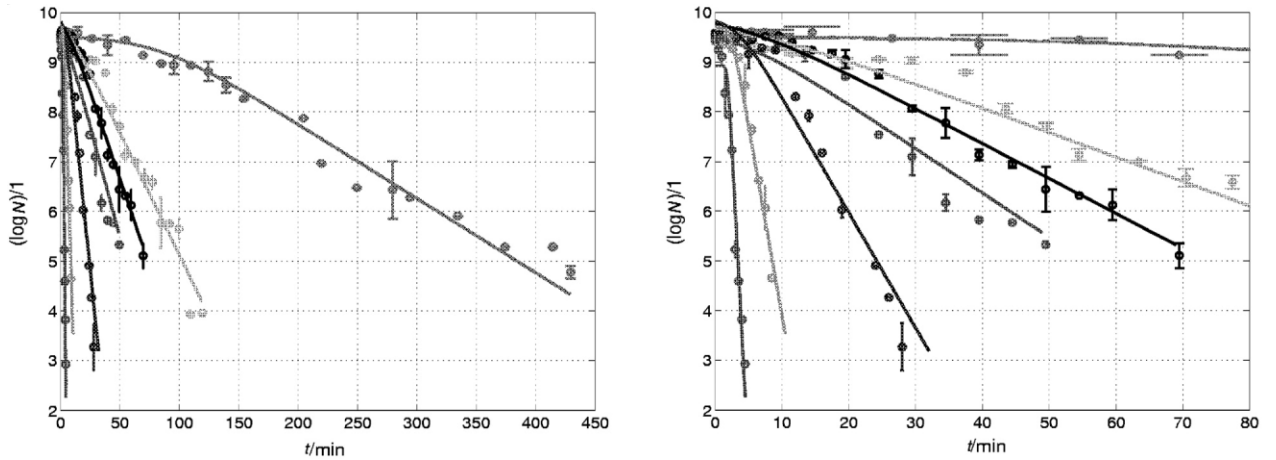

Fig. 2. Global identification of parameters *a, b, c, d*. Full line: model fit of inactivation model (Eq. 8). Circles: Raw data and 90 % confidence intervals (CI) (for data where duplicates are available). Right plot: detailed figure for a time range of 0–80 min. The fitting capacity of the model is R^2_{adj}=0.96

$R^2_{adj} = 1 - \frac{n-1}{n-p} \cdot \frac{SSE}{SSTO}$, where n and p are number of data points and number of parameters, respectively, whereas *SSE* and *SSTO* are the sum of squared errors and the total sum of errors (*23*).

Secondly, a global identification is chosen for estimating the parameters *a, b, c, d* making use of all inactivation curves in one parameter estimation step (Fig. 2), aiming at prevention of the accumulation of fitting errors (as elaborated in previous works (*15,26–28*)). Therefore, the functions of S_l and k_{max} (Eqs. 6 and 7) are incorporated into the primary model (Eq. 4) and *a, b, c, d* are estimated using all (t,T,$N(t)$) measurements:

$$\log(N(t)) = \log(N(0)) - \frac{e^{(c\cdot T-d)}\cdot t}{\ln(10)} + \frac{e^{(c\cdot T-d)}\cdot\left(\frac{a}{T-b}\right)}{\ln(10)} - \\ - \log\left(1+\left(e^{\left(e^{(c\cdot T-d)}\cdot\left(\frac{a}{T-b}\right)\right)}-1\right)\cdot e^{\left(-e^{(c\cdot T-d)}\cdot t\right)}\right) \quad /8/$$

Results are reported in Table 2. Inserting the obtained parameter estimates, *i.e. a, b, c, d* in Eq. 5, the identified function of t_{xD} then becomes:

$$t_{xD}(T) = S_l + x \cdot AsymD = \frac{16.22}{T-51.80} + \frac{x\cdot\ln(10)}{e^{(0.60T-34.36)}} \quad /9/$$

The developed mathematical approach was used to perform simulations for different number of log cycles for a range of different, constant temperatures. In Fig. 3 the hyperbolic shape of Eq. 9 is depicted. When using Eq. 9 to calculate a t_{xD} at certain T, the standard error of the computed results is calculated based on the error propagation technique (*29*).

Table 2. Estimated microbial parameters and their standard errors using the integrated primary inactivation model (Eq. 8)

a	*b*	*c*	*d*
16.22±7.29	51.80±3.47	0.60±0.02	34.36±1.23

Discussion

The following statements are to be made to interpret the behaviour of the developed Eq. 9 and Fig. 3:

(*i*) As T lowers to 51.8 °C Eq. 9 becomes undetermined and t_{xD} approaches infinity. This means that the initiation of the microbial inactivation of *Escherichia coli* K12 for BHI broth conditions should be expected at a temperature of (51.8±3.47) °C. Parameter *b* could be denominated as »the reference minimum temperature for inactivation«. Although the standard error has a high value, *i.e.* 3.47, the reference minimum temperature should be lower than 52 °C (temperature at which inactivation is achieved experimentally). The above observation for parameter *b* corresponds to previous studies in the literature. Thermal inactivation experiments of *E. coli* K12 at 49.5 °C (following the same protocol for the data generation as in this case study) have shown a long shoulder length, S_l, of 180 min (*13*). Additionally, all the ICMSF reports for inactivation of *Escherichia coli* are at temperatures ≥50 °C (*30*). Better defining of this temperature boundary requires (*i*) more inactivation experiments near the boundary value, and (*ii*) experimental studies in which different influencing factors are taken into account, *e.g.* pH, water activity, fat content, *etc.*

(*ii*) When T takes (very) high values, t_{xD} tends to zero. Considering that the highest temperature in food processing is normally around 135 °C, *e.g.* the case of UHT (ultra high temperature processing), the time for achieving 9 log reductions, *i.e.* (t_{9D}), acceptable for »commercial sterilization« would be (according to Eq. 9) 11.70 s (with a standard error SE=5.22 and a 90 % confidence interval, CI=8.66). Consequently, an engineer could choose a process of 20.36 s (upper confidence limit) in order to have a confidence of 90 % for achieving the processing target. These calculations largely fall outside the microbial experimental region (as the maximal temperature used in this research is 60.6 °C) and should be taken with caution.

(*iii*) Another example illustrating a possible industrial applicability of the developed approach can be the pasteurization at 60 °C. Assume, for instance, an engi-

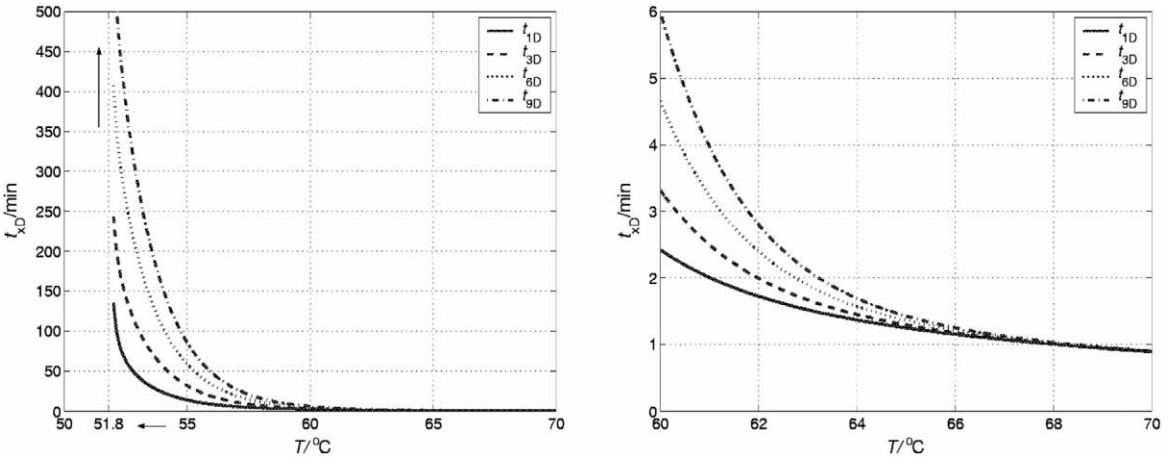

Fig. 3. Simulations of Eq. 9 for 1, 3, 6 and 9 log cycles of reduction of *E. coli* K12 with respect to inactivation temperature. Reading key: if a constant processing temperature of 55 °C is chosen, t_{1D}, t_{3D}, t_{6D} and t_{9D} equal 14.04, 31.98, 58.89 and 85.81 min, respectively. Right plot: detailed figure for a temperature range of 60–70 °C

neer who wants to obtain an increased safety, shelf-life and microbiological quality of his product by pasteurization. If he considers that his target microorganism is *E. coli* and the process that he wants to assess is at a temperature of 60 °C, then based on Eq. 9 the time for achieving 7 log reductions (*i.e.* the lowest reduction level of the current practice in pasteurization of non-sporing Gram-negative bacteria in different menstrua (*16*)) will be 5.10 min (SE=1.22, 90 % CI=2.02). Therefore, the engineer could choose a process of 7.12 min (upper confidence limit) for achieving the processing target.

Conclusions

A generic approach to quantify the microbial heat resistance through a t_{xD} concept based on non-log-linear inactivation curves is the main outcome of this research. Apart from its applicability in predictive microbiology, the new model approach can be useful in the field of food safety management through the FSO concept. FSO is the maximum frequency and/or concentration of a microbial hazard in a food considered tolerable for consumer's protection, but which does not specify how an operator achieves compliance (*3*,*4*). As the FSOs define the level of control that is expected for a food operation, then it can potentially be met through the application of the introduced performance and process/product criteria by using the concept of t_{xD}.

The developed approach will be further validated for different pathogenic microorganisms of high interest for the food industry. Additionally, the possibility to describe the survival of low numbers of microbial populations by using non-linear microbial modelling approaches that describe tailing next to shoulder features (as in the original formulation of the models (*7*)), where probabilistic effects become important, are of interest as well. Studying the tailing phenomenon can be considered as a further step for validating the model structure (Eq. 9). In a recent workshop of IDF, International Dairy Federation, (May 5–8, 2003, Kiel, Germany) entitled »Revisiting heat resistance of microorganisms in milk« the technological impact of tailing in non-linear survivor curves was discussed (*31*). According to the working group: (*i*) if the level of population that survives in the tail is independent of the initial microbial concentration, the tailing results from an experimental artifact (which has to be searched for and dealt with); (*ii*) if the tailing is dependent on the initial level, then it should be searched if the tailing effect also appears in the process in the real world and not only in the lab; (*iii*) if it is also a phenomenon appearing in the real world, then it is considered a safety issue; (*iv*) if it is a safety issue, it should be quantified in order to change the process in such a way that the tailing disappears.

In conclusion, the t_{xD} concept and the secondary models developed in this research are in line (with respect to the use of different model structures and parameters) with the recent research. IFT research summit resolution concluded the following:

»Since there is significant evidence that microbial survivor curves can be described by non-log-linear kinetic expressions, the scientific and technical community should recognize alternative models and parameters for description and communication of the survival of microbial populations when exposed to various lethal agents« (*9*).

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Alternativni pristup »ne-log-linearnoj« toplinskoj inaktivaciji mikroorganizama – modeliranje smanjenja broja logaritamskih ciklusa ovisno o temperaturi

Sažetak

Na osnovi koncepta smanjenja broja logaritamskih ciklusa razvijen je matematički model koji obuhvaća učinak zaštitnih sastojaka u mediju oko ili unutar svake stanice (shoulder effect) tijekom kvantitativne toplinske inaktivacije mikroorganizama. Soj bakterije *Escherichia coli* K12, od prije poznat po otpornosti na toplinu, upotrijebljen je za kvantitativno generičko i točno mjerenje u BHI hranjivoj podlozi, određivanjem vremena t potrebnog za logaritamsko smanjenje (log x) mikrobne populacije, tj. određivanje t_{xD}, kao funkcije primijenjene temperature T. Dobiveni su podaci o preživljavanju istraživanoga mikroorganizma u rasponu temperatura od 52 do 60,6 °C. Dužina zakrivljenoga dijela krivulje (shoulder length) S_l i specifična brzina inaktivacije k_{max} izvedene su iz matematičkog izraza koji opisuje »ne-log-linearno« ponašanje. Temperaturne ovisnosti S_l i k_{max} upotrijebljene su za oblikovanje funkcije $t_{xD}(T)$. Određivanje $t_{xD}(T)$ parametara tijekom cijeloga postupka identifikacije živih stanica dopušta pouzdano predviđanje vremena u kojem bi se postiglo potrebno smanjenje broja mikroorganizama. Jedan od parametara $t_{xD}(T)$ funkcije predložen je kao smjernica za minimalnu temperaturu inaktivacije. Za razmatrano istraživanje ustanovljena je vrijednost od 51,80 °C (sa standardnom pogreškom, SP=3,47). Na kraju je ustanovljeno vrijeme učinkovite industrijske sterilizacije i pasterizacije za istraživani proizvod, tj. BHI hranjivu podlogu, od 11,70 s (SP=5,22), odnosno 5,10 min (SP=1,22). Uvažavajući nesigurnost (temeljenu na 90 % intervala pouzdanosti, IP) dokumentirano sigurni postupak za ova dva procesa zahtijeva 20,36 s, odnosno 7,12 min.